



Temperature and Salinity Effects on Bioaccumulation, Gill Structure, and Radiation Dose Estimation in the Milkfish *Chanos chanos* Exposed to ^{137}Cs

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ABSTRACT

The present trend of global warming has led to an increase in seawater temperature and salinity. The effects of increasing salinity and temperature on the accumulation of ^{137}Cs by milkfish *Chanos chanos* was studied under laboratory conditions to obtain information on *Chanos chanos* adaptability under environmental changes. The uptake of radioactive cesium by *Chanos chanos* increased with temperature of seawater. The concentration factors (CF) of ^{137}Cs for temperatures of 25°C, 27°C, 29°C, and 31°C at steady state period were 5.25, 5.91, 6.78, and 9.98 mL g⁻¹ for the whole-body of *Chanos chanos*. The concentration factors at steady state (CF_{ss}) of ^{137}Cs for salinities of 26‰, 29‰, 32‰, and 35‰ were 6.23, 9.93, 9.24, and 6.86 mL g⁻¹, respectively. After temperature exposure to 31°C, the fish gills showed hyperplasia of epithelial cells in branchial secondary lamellae, congestion of blood vessels, and hypertrophy of pillar cells. The fish from the treatment group exhibited hemorrhage between the branchial secondary lamellae and an abundance of mucous substance in comparison with control group. This study links radionuclide bioaccumulation data and monitoring data obtained in the field and laboratory experiment with radiation dose determined by ERICA Tools, an approach that will enable better linkages to be made between exposure and dose in *Chanos chanos* and its marine food web.

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INTRODUCTION

Development of nuclear facilities, including nuclear power station in Indonesia, creates a possibility of a release ^{137}Cs within allowable level into aquatic environment [1]. The radionuclide ^{137}Cs is a fission product which may be present in the low-level aqueous radioactive waste discharged from nuclear facilities. Cesium is of considerable environmental concern because of its high mobility in the marine environment and its ability to accumulate in marine organisms. Understanding the

biological fate and transport of cesium in aquatic environment is essential for a sound risk assessment of marine ecosystem.

Marine ecosystems are very important in the regulation of the climate, and they are very sensitive to climate change [2]. The predicted impacts of climate change on marine ecosystems can directly and indirectly affect species abundance and distributions, habitat quality, community composition, and changes in population dynamics [2].

The international organization of climate change, the Intergovernmental Panel on Climate Change (IPCC) in its most recent assessment reported that on a global scale, over the period of

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1971 to 2010, the upper 75 m of the ocean warmed by 0.11°C per decade. The gradual rise in ocean temperature initiates episodes of water evaporation. These two indicators of global warming affect two physical marine parameters, temperature and salinity.

Studies concerning the likely impact of this warming on marine habitats have been limited. Previous study investigated the effects of temperature [3-5] and salinity [6-8] on aquatic organisms. Moreover, climate change was observed to influence fish reproductive and growth hormones and alter gill structure and genes [9-11]. On the other hand, there have been few studies that investigated how temperature or salinity could affect accumulation of contaminants, especially radioactive contaminants, by marine organisms. Labarthe *et al.* [12] showed that the increase of temperature and pCO₂ as an effect of global warming impacts the bioaccumulation of radionuclides and trace elements in the eggs of *Sepia officinalis*. The study did not comprehensively discuss the effects of temperature on juvenile stage.

The milkfish *Chanos chanos* is one of the earliest fish species to be domesticated for food. Studies on the accumulation of radioactive substances by juvenile *Chanos chanos* have been very limited, especially the ones focusing on the effects of temperature and salinity changes as impacts of global warming. Prihatiningsih *et al.* (2016) observed that the accumulation of ¹³⁷Cs on *Chanos chanos* increased when the body weight increased. Unfortunately, this study did not consider the effects of environmental factors such as salinity and temperature changes as impacts of global warming. Additionally, a particular investigation that is missing from the few previous studies of radionuclide bioaccumulation in fish is the connection between exposure, capability of accumulation, and delivered dose. The use of the ERICA Tool as a new technique in dosimetry modeling and dose estimations can now be applied to extending the results of bioaccumulation investigation to estimating the radiation dose received by fish and other biotas exposed to radionuclides [13,14]. However, the application of the ERICA Tool to estimate the time variation of dose rates for fish exposed to radionuclides through multiple routes of exposure has not been conducted.

Further research on target organ as an early damage indicator in fish is also still needed. The gills, which participate in many important functions in fish, such as osmoregulation, respiration, and excretion, are particularly sensitive to changes in water quality as they remain in close contact with the external environment [15].

Changes in temperature, salinity, concentration of heavy metals, and acidity, and other changes in the composition of the environment, as well as other physical and chemical alterations of the aquatic medium, influence the fish gill as an sensitive organ. Heavy metal and acid pollution has been reported to induce desquamation to primary lamellar epithelium and epithelium hyperplasia and alter cell structure [15,16]. Moreover, changes in temperature and salinity have also been reported to alter the gill structure in tropical reef fishes [4].

Our objective was to investigate the uptake of ¹³⁷Cs in juvenile *Chanos chanos* exposed via seawater. Data on ¹³⁷Cs accumulation and its concentration in seawater and sediment is useful for risk assessment in Jakarta Bay. In addition to modeling, we used ERICA Tool to estimate dose rates and accumulated doses received by marine organisms, including *Chanos chanos*, to link radionuclide exposure with dose. Moreover, we also investigated how temperature can affect the structure of gills as sensitive organs dealing with thermal changes in the environment.

EXPERIMENTAL METHODS

Organisms collected

Chanos chanos (lengths of 3.2 to 5.8 cm) were collected from a fish farm in Tanjung Pasir, Jakarta Bay, Indonesia, in March 2015. Afterwards, they were shipped to the Marine Radioecology Laboratory, Center for Technology of Radiation Safety and Metrology in South Jakarta, where they were acclimatized to laboratory conditions. The *Chanos chanos* were gradually acclimatized to experimental conditions, including temperature (25°C, 27°C, 29°C, and 31°C) and salinity (26‰, 29‰, 32‰, and 35‰) for one month prior to the experiment. During this period, the *Chanos chanos* were fed daily with LJA-3 commercial pellets.

Radiotracers and counting

The uptake kinetics of ¹³⁷Cs was determined by using high specific activity radiotracers purchased from Polatom, Poland (¹³⁷Cs as CsCl, t_{1/2} = 30 years). The radioactivity of the tracers was measured nondestructively using an NaI gamma detector (Bicron type 3428) at 661.66 keV connected to a multi-channel analyzer and a computer equipped with Genie-2000 spectra analysis software. Standard sources of known

activities and appropriate geometries were used to determine the radioactivity by comparison.

^{137}Cs uptake experiments were conducted using six individual animals held in 10 l for each temperature and salinity. Animals of similar size were used to minimize the possibility of size-related differences in radioisotope uptake. The nominal activity of the ^{137}Cs radiotracer was 2 kBq/l. The seawater was renewed daily during the first week, then it was changed every second day in order to keep the radioactivities in the seawater at a constant level. The activity of the radiotracers in the seawater was checked daily to calculate the time-integrated activities of the tracer [17]. The *Chanos chanos* were counted for 15 minutes.

Kinetics

The uptake of the radiotracers from water was expressed as a change in the concentration factor (CF). The CF of ^{137}Cs is defined as the ratio of the activity per unit mass in the fish (Bq g^{-1}) to the activity per unit volume in the water (Bq mL^{-1}), which is calculated as the mean before and after the exposure for each time point. The uptake rate was calculated as the slope of the linear regression line between the CF and the time of exposure multiplied by the concentrations of dissolved Cs. The uptake kinetics was described by using a single-component first-order kinetic model [17]:

$$CF_t = CF_{ss}(1 - e^{-k \cdot t}) \quad (1)$$

where CF_t and CF_{ss} represent the concentration factor at time t (in days) and the steady state concentration factor, respectively, and k is the rate constant (d^{-1}).

Gill histology

Almost similar with ^{137}Cs uptake experiment, the examined fish were divided into two groups (two samples for each group). The control group was left under normal conditions to study the normal structure of gills, while the second group, or the treatment group, was exposed to high temperatures of up to 31°C for 24 h daily for 10 days. The gills of the test fish were excised, keeping the rakers and the filaments intact. Those gills were then rinsed in normal saline, fixed in 10% formalin for about 24 h at 4°C , then dehydrated in an ethyl alcohol series of ascending concentrations, cleared in xylene, infiltrated with paraffin at 56°C , then embedded in paraffin wax [18]. Thin sections of the selected gill tissues of

about $4 \mu\text{m}$ thickness were cut by means of a rotatory microtome, dehydrated, and stained with haematoxylin and eosin (H&E), and mounted with di-n-butyl phthalate in xylene (DPX). The sections were examined and photomicrographed using an Olympus microscope fitted with photographic attachment. The prepared slides were used to describe the normal histological structure and histological alterations in the gills.

RESULTS AND DISCUSSION

The uptakes of ^{137}Cs under different temperatures displayed exponential kinetics reaching a steady state (Fig. 1 and Table 1). The CF of ^{137}Cs for temperatures of 25°C , 27°C , 29°C , and 31°C at steady-state conditions were 5.25, 5.91, 6.78, and 9.98 mL g^{-1} in whole-body fish, respectively. For different salinities, the uptakes of ^{137}Cs are shown in Fig. 2 and Table 1. The CF_{ss} of ^{137}Cs for salinities of 26‰, 29‰, 32‰, and 35‰ were 6.23, 9.93, 9.24, and 6.86 mL g^{-1} , respectively.

Table 1. *Chanos chanos* concentration factor exposed to ^{137}Cs for different temperatures and salinities

Temperature ($^\circ\text{C}$)	Concentration Factor (mL g^{-1})	Salinity (‰)	Concentration Factor (mL g^{-1})
25	5.25 ± 0.26	26	6.23 ± 0.31
27	5.91 ± 0.30	29	9.93 ± 0.50
29	6.78 ± 0.34	32	9.24 ± 0.46
31	9.98 ± 0.50	35	6.86 ± 0.34

The results of the present study demonstrate temperature-enhanced initial uptake rates of ^{137}Cs from water. Increases in accumulation rates at higher temperatures may be partially due to amplified metabolic activity. Animals were observed to be much more active at 31°C than at 25°C and the increased internal circulation of water through the digestive systems and respiratory may result in enhanced in the amounts of all elements bound to internal body surfaces. In addition, production of metal-binding proteins or external mucus may be greater at elevated temperatures.

Our study indicated that the Cs uptake was considerably affected by ambient salinity, consistent with many previous observations on the effects of salinity on bioaccumulation [19]. The concentration factor of cesium increased with salinity at the salinity range of 26 to 29‰ and started decreasing with salinity from 32 to 35‰. This study observed that the optimal salinity for bioaccumulation in *Chanos chanos* was 29‰. Various mechanism have been proposed for the underlying salinity effects,

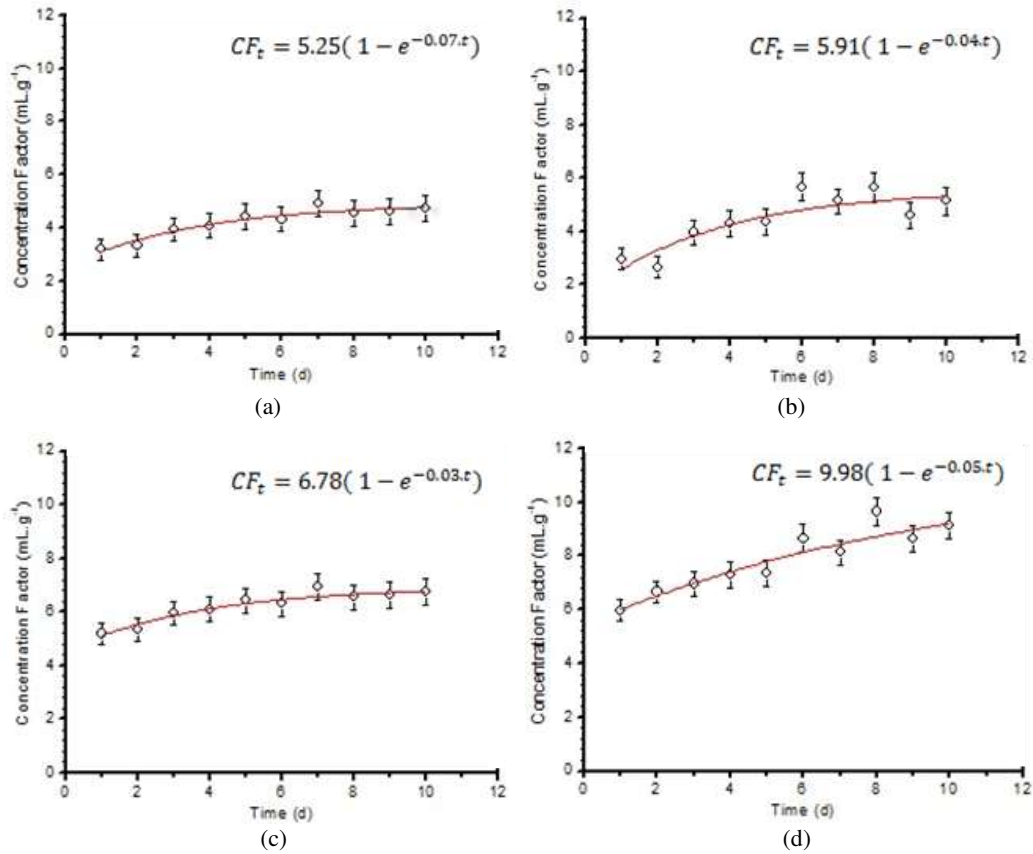


Fig. 1. Uptake kinetics of ^{137}Cs by *Chanos chanos* in different seawater temperatures (a. 25°C, b. 27°C, c. 29°C, d. 31°C).

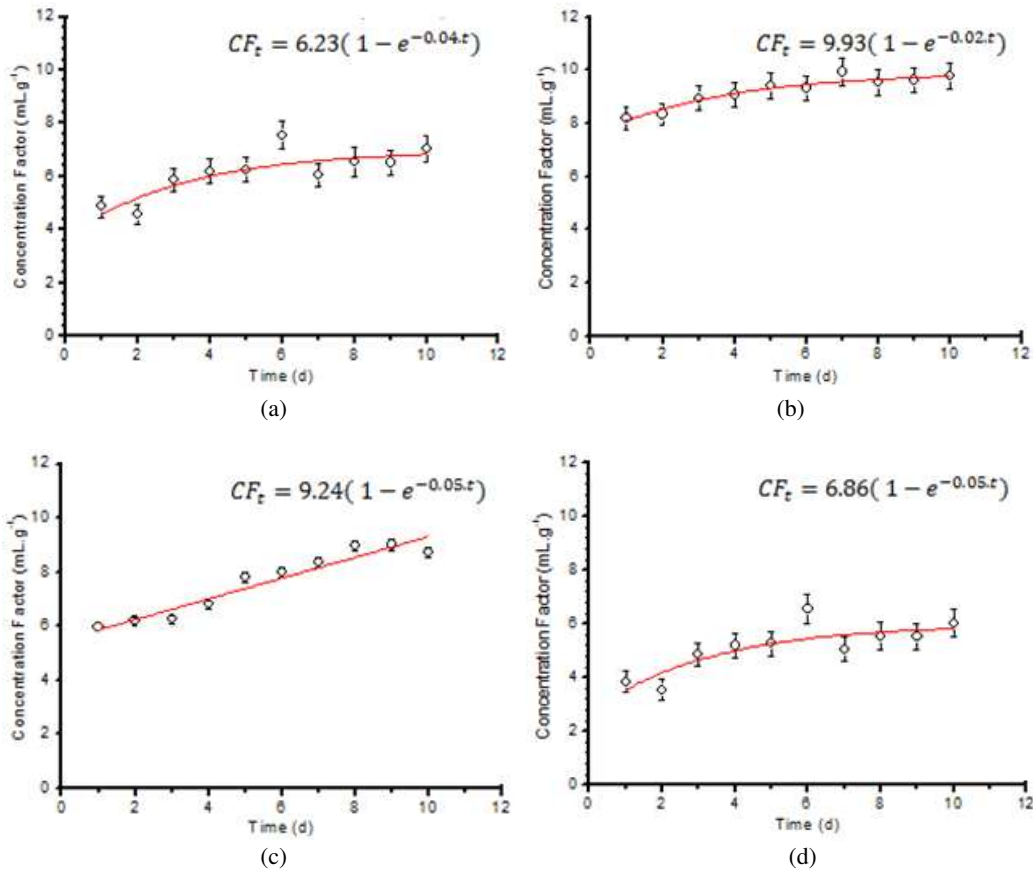


Fig. 2. Uptake kinetics of ^{137}Cs by *Chanos chanos* in different seawater salinities (a. 26‰, b. 29‰, c. 32‰ and d. 35‰).

including the physiological conditions of the *Chanos chanos* such as respiration rate, or change in cell volume and permeability of tissue. Wang (2000) demonstrated that the variability of cesium uptake at different salinities was primarily due to a change in ambient K^+ concentration. Further studies are needed to determine the influence of K^+ related to salinity effect on *Chanos chanos* bioaccumulation.

The main project of ERICA Integrated Approach is the quantification of risk or risk assessment to the environment for a given release of radioactivity. To attain this aim, the data on environmental dosimetry and transfer characteristics of radioisotopes in the environment are combined to provide an estimate of exposure. Furthermore, the ERICA Integrated Approach recommends the use of expected values (or best estimates) of activity concentrations. The assessment chronology in this study uses normal ^{137}Cs activity concentration in seawater and sediment [20]. On other hand, the distribution coefficient (K_d), CF, radiation weighting factor, and uncertainty factor in each organism were taken from secondary data which is prepared by ERICA. The screening dose was set at $10 \mu\text{Gy}\cdot\text{h}^{-1}$.

The concentration factor for cesium is a critical value in a risk assessment study of the environmental effect of cesium in coastal and offshore environments. Additional concentration factor data is needed to better describe the distribution of cesium concentration factors in the edible portions of animals used for food. Generic concentration factors are appropriate for use only in a screening-level assessment.

The results of the risk analysis in *Chanos chanos* and other organisms in marine food web are listed in Table 2. These results indicated that all of the total dose rates for all organisms studied were lower than the screening dose rate (10 mGy/h). Based on the results from the Tier 2 assessment (Table 2), it is concluded with confidence that there will be no impact on *Chanos chanos* and other organisms in the food web because all calculated doses listed in Table 2 are lower than the $10 \mu\text{Gy/h}$ screening level at Tier 2. Given the results shown in Table 2, if ^{137}Cs release actually occurs with activities close to the environmental monitoring data used here, the ERICA Tool would suggest that further investigations are not warranted. A range of decisions will then need to be taken, based on the suggestions given in the generic ICRP exposure situations [13].

Environmental conditions can result in compensatory mechanisms that deal with environmental stressors, as in cellular hyperplasia, and in direct toxic effects of the pollutants leading to necrosis and degeneration [15]. In the present case, it seems that the results are in the form of compensatory mechanisms. All histological lesions observed in the

gills of *Chanos chanos* in this study, are categorized under cellular atrophy, lamellar cell hyperplasia and hypertrophy, hemorrhage and bloody congestion, lamellar damage, and lamellar fusion; these results could be linked with increase in the activities of the test organisms exposed to the changing habitat temperature.

Table 2. ERICA Tool tier-2 assesment for total dose rate

Organism	External	Internal	Total Dose rate	Screening Value
	^{137}Cs ($\mu\text{Gy h}^{-1}$)	^{137}Cs ($\mu\text{Gy h}^{-1}$)	^{137}Cs ($\mu\text{Gy h}^{-1}$)	^{137}Cs ($\mu\text{Gy h}^{-1}$)
Benthic fish	1.82×10^{-5}	1.74×10^{-5}	1.99×10^{-4}	10
Benthic mollusc	1.94×10^{-4}	9.15×10^{-6}	2.03×10^{-4}	10
Crustacean	1.76×10^{-4}	1.16×10^{-5}	1.87×10^{-4}	10
Macroalgae	2.06×10^{-4}	1.64×10^{-5}	2.16×10^{-4}	10
Pelagic fish	3.54×10^{-7}	1.84×10^{-5}	1.88×10^{-5}	10
Phytoplankton	4.92×10^{-7}	6.95×10^{-7}	1.19×10^{-6}	10
Zooplankton	4.27×10^{-7}	1.90×10^{-5}	1.95×10^{-5}	10
<i>Chanos chanos</i>	8.24×10^{-8}	3.73×10^{-7}	4.55×10^{-7}	10

After exposure to 31°C temperature, the fish gills showed hyperplasia of epithelial cells in branchial secondary lamellae, congestion of blood vessels, hypertrophy of pillar cells (Fig. 4), abundance of mucous substance, and hemorrhage between the branchial secondary lamellae (Fig. 5), in comparison with control group (Fig. 3).

Under high temperatures, cellular hypertrophy and hyperplasia are an important feature. Cells are principally derived from the primary lamellae; the cells then migrate distally, resulting in an accumulation of cells at the leading edge of the secondary lamellae [16,21]. As a result, this could contribute to an increase in the diffusion distance from surrounding water to capillaries and simultaneously increase in the amount of tissue in the secondary lamellae and impair the diffusion of oxygen through the swollen epithelium [16,22]. Moreover, the gills showed bloody congestion. Some changes in blood vessels may also occur when fishes suffer a more severe type of stress. In this condition, damaged pillar cells can result in an increased blood flow inside the lamellae, causing dilation of the marginal channel, bloody congestion, or even aneurysm [21]. The rupture of the gill epithelium could effect heamorrhage; this lesion can be understood as a reflection of the direct action of the thermal agent on the tissue [4]. The mucus-packed cavities observed in the gill filaments of *Chanos chanos* may be considered as an ion trap to concentrate trace elements from surrounding water and favor cell adhesion between the neighbouring secondary lamellae [22].

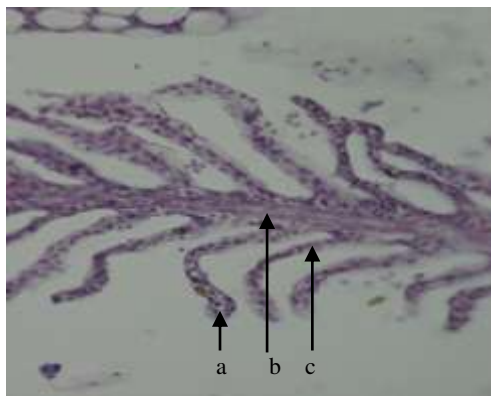


Fig. 3. Gill section from control *Chanos chanos* showing epithelial cells (a), secondary lamellae (b), and primary lamellae (c). H&E. $\times 40$.

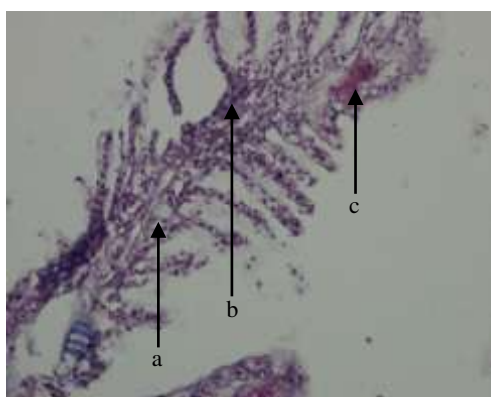


Fig. 4. Gill section of *Chanos chanos* exposed to 31°C temperature showing hyperplasia of the epithelial cells (a), hypertrophy of the pillar cells (b), and blood congestion (d). H&E. $\times 40$.

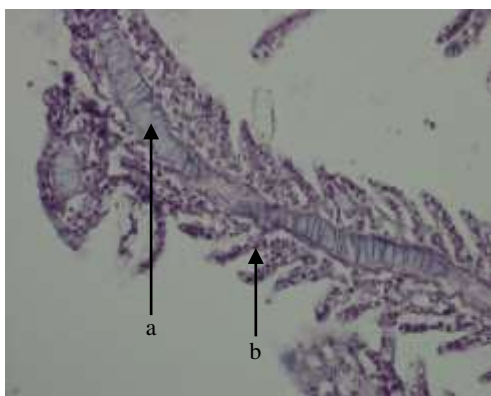


Fig. 5. Gill section of *Chanos chanos* exposed to 31°C temperature showing structure change in primary lamellae (a) and epithelium rupture with hemorrhage (b). H&E. $\times 40$.

Comprehensive studies that relate the impacts of climate change is greatly needed in the future, as climate changes as mentioned above potentially result in the decrease of ocean pH due to elevated CO₂ concentrations; this could also induce alterations in morphology of gill to keep ion/osmotic and gas exchange balance. This possibility should be considered in future studies.

CONCLUSION

Environmental factors, including salinity and temperature, affect the metabolism of organisms and their growth and consequently their uptake of cesium. Generally, small increases in salinity and temperature tend to increase the cesium uptake and biological activity. The CF_{ss} of ¹³⁷Cs for temperature 25°C, 27°C, 29°C, 31°C were 5.25, 5.91, 6.78, and 9.98 mL g⁻¹ in the whole body of *Chanos chanos*. The CF_{ss} of ¹³⁷Cs for salinities of 26‰, 29‰, 32‰, and 35‰ were 6.23, 9.93, 9.24, and 6.86 mL g⁻¹, respectively. Optimum temperature and salinity for bioaccumulation process of ¹³⁷Cs by *Chanos chanos* were 31°C and 29‰. After exposure to 31°C temperature, the fish gills showed hyperplasia of epithelial cells in branchial secondary lamellae, congestion of blood vessels, and hypertrophy of pillar cells. The exposed fish exhibited hemorrhage between the branchial secondary lamellae and an abundance of mucous substance in comparison with control group. From ERICA assessment, it was found that the total dose rates for *Chanos chanos* other organisms in the marine food web and were lower than the screening dose rate (10 mGy/h), which means that there will be no impact on the *Chanos chanos* and other organisms in food web because the ¹³⁷Cs concentration was within the allowable level.

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